Vol. 4, Nos. 7-12 July-December 1928 Nos. 46-51

Bulletin of the Agricultural Chemical Society of Japan.

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Published by the
Agricultural Chemical Society of Japan.
C/O Faculty of Agriculture, Tokyo Imperial University.

Single Copy (Postage inclusive) :- ¥ 0.35
Annual Subscription (12 numbers) :- ¥ 3.50

The Agricultural Chemical Society of Japan.

President : Keijiro Aso.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Editor : Kijiro Aso.
Associate Editors : Kakuji Gotō and Yoshihiko Matsuyama.

ON THE ORIGIN OF ALDEHYDES IN FERMENTATION PRODUCTS. PART V.

By

MASAKAZU YAMADA.

(Received June 20th., 1928.)

If we search the components of extract for the mother substances of aldehydes which come by heating from the distilling–residue of several fermentation products,(1) oxy-acids, amino–acids or sugars may first be brought to mind.

It is well known(2) or can easily be proved by Rimini's test that a part of lactic acid is decomposed by heating itself or in aqueous solution into acetaldehyde.

Leucic acid corresponding to isovaleraldehyde was not found in saké though about 10L. was treated.

From amino–acids ordinarily amines are produced, if they are heated themselves, but when treated together with sugars, melanoidin was formed(4) and at the same time production of a flavour like the roast malt(5) or Shōyu (6) was perceived. Moreover in that case production of aldehydes from the mixture of glucose, amino-acid and glycerine——acetaldehyde from d–l–alanine, isovaleraldehyde from l–leucine and phenylacetaldehyde from l–phenylalanine——was lately verified by S. Akabori.(7)

The author distilled quietly the mixture of sugar and amino–acid in aqueous solution on the oil-bath of 120∼140° so as to equalize the condition with the case of distilling the extracts and also with the same care as Neuberg and Kobel's for their study on the reaction between methylglyoxal and amino–acids(8); and obtained almost the same results as Akabori's or Neuberg and his co–worker's. Thus, from the mixtures of sugar (2 parts), —glucose, fructose, galactose, maltose, saccharose, lactose and arabinose— d–alanine (1 part) and water (2–5 parts) acetaldehyde was obtained in every case

---

(1) M. Yamada: Bull. Agri. Ch. Soc. Japan. **4**, 18—21, 1928.

(2) Erlenmeyer: Z. **343**, 1868.

(3) Drechsel: Ber., **25**, 3503.

(4) L. C. Maillard: Compt. Rend. **154**, 66—8, 1912.

(5) C. J. Lintner: Woch. f. Brau. **30**, 51—5, 1913.

(6) K. Kurono and J. Fukai: Report of Institute of Brewiny, Tokyo, **97**, 1—75, 1928.

(7) S. Akabori: Proc. Imp. Acad. Tokyo., **3**, 672—4, 1927.

(8) C. Neuberg u. M. Kobel. Bioch: Zs., **185**, 477—9, 1927; **188**, 197—210, 1927.

(Rimini's test), but no acetaldehydes arose from alanine or sugars alone.

The reaction-products from the mixture of several amino-acids or their salts and glucose in the aqueous solution are tabulated below.

In each case 10c.c. of water was supplied several times, when the content of the flask reached about 10c.c.

	amino acid	glucose	water (original)	water (supplemented)	in the distillate		
					Rimini's test	furfurol react.	remarks
	g.	g.	c.c.	c c.			
glycocoll	3	10	30	50	–	+	Rimini's test for formald. negative
d-alanine	5	10	50	40	⧻	+	× p-nitro-ph. M P. 127°
l-leucine	3	15	30	60	±	+	* p-nitro-ph. M. P. 109°
glutamic acid-Na	10	20	50	160	+	+	p-nitro-ph was not obtained.
aspartic acid-Na	2	10	30	100	⧻	±	× p-nitro-ph. M. P. 128°
asparagine	2	10	30	60	⧺	+	× 〃 M. P. 126°
cystine	1	5	100	50	purple	+	〃 yellow needle
d-l-phenyl-alanine	1	5	20	50	–	+	♯ Semicarb. M. P. 154°
Tyrosine	5	25	25	50	±	+	——

Positive Rimini's test in leucine and tyrosine may perhaps be due to the products from some impurities.

× M. P. of the mixture with the pure acetald.-p-nitrophenyl hydrazone (M. P. 128°) shows no depression.

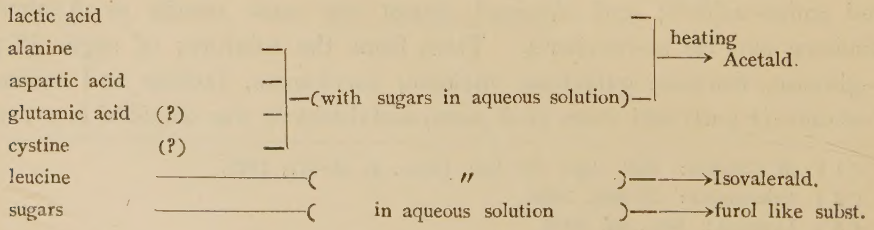
* The same as above with the pure isovalerald.-p-nitroph. (M. P. 110°)

♯ Phenyl acetald.-semicarbazone M. P. 152°

Glycocoll does not give the expected product formaldehyde and products from tyrosine is not yet clear.

The furfurol reaction in all cases may be due to the direct decomposition product of glucose and the fact is important for explaining an origin of furfurol like substance in several fermentation products.

Now the origins of aldehydes from the extracts by heating will be summerized as follows.



Addendum :-

The valeraldehyde in the previous paper part III will be somewhat crude isovaleraldehyde because its derivatives (fract. 7 in the case of Shōyu) showed following data after perpetual recrystallization.

	p-nitroph. M.P.	aldomedon M.P.	aldomedon-anh. M.P.
Fract 7	107°	149°	164°
isovalerald.	110°	153°	167-9°
mixture of above-two	109°	151°	—

SYNTHESES OF OPTICALLY ACTIVE COMPOUNDS BY YEASTS. I.

SYNTHESIS OF L-ASPARTIC ACID FROM FUMARIC ACID.

By

YUSUKE SUMIKI.

(*Agricultural chemical laboratory, Tokyo Imperial University*)

(Received June 25th., 1928)

Preparation of the beer yeast.

The beer yeasts were washed ten times by decantation with 1 normal solution of sodium chloride. They were filtered and suspended in 1 normal solution of sodium chloride or in distilled water. This suspension was finally aerated for 2 hours and was then ready for use. It was stored in the refrigerator. Although it was generally used fresh, there was no marked change in its activity for at least 2 months.

Procedure of synthesis.

The reaction mixtures, containing known concentrations of sodium fumarate, ammonium chloride and Clark & Lubs's phosphate buffer (PH 6.8 or 7.0), with the beer yeast to be tested were allowed to stand in the thermostat (37∼45°) and shaked once a day. The experiments were done aerobically or anaerobically.

Isolation and identification of l-aspartic acid.

The reaction mixture was filtered, evapolated to a small quantity and added with hydrochloric acid until the congored paper turned its colour into blue. After extraction with ether, it was neutralized with ammonia, added with saturated solution of copper acetate and heated for half an hour in the boiling water bath. After filtration to remove the light blue precipitate, the clear deep blue filtrate was allowed to stand in the refrigirator for 3 days, when crystallisation of the copper aspartate was completed. The crystals were filtered off, well washed with cold water and dried to constant weight over sulphuric acid in the desccator. Free l–aspartic acid was obtained by treating the copper salt with H_2S and recrystallized from hot water, decolorzing if necessary with a little animal charcoal.

0.1740g. subst. gave 0.0822g. H_2O and 0.2296g. CO_2.
0.0652g. subst. gave 6.40 cm.³ N_2 (745.0 mm. 27.5°C).
Calculated for $C_4H_7O_4N$ C 36.09% H 5.26% N 10.53%
Found C 35.98% H 5.24% N 10.54%

0.6606 g. of aspartic acid was dissolved in 17.3488 g. of hydrochloric acid (sp. gr. 1.050).

$$\alpha = +0.900^\circ,\quad l = 1\text{dm.},\quad d = 1.0865,\quad p = \frac{0.6606 \times 100}{17.3488 + 0.6606}$$

$$\therefore\ [\alpha]_D^{25^\circ} = +22.83^\circ$$

The ether extract of the reaction mixture was evapolated and dried to constant weight in the steem bath. After weighting, each melting point was observed in a closed capillary tube without recrystallisation. In one case it melted between 183° and 185° and another case it melted between 278° and 280°. So it is clear that succinic acid and fumaric were isolated.

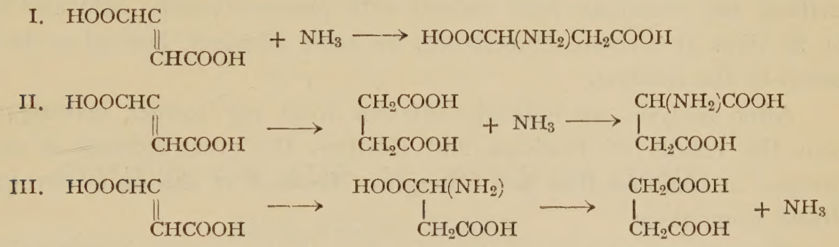
No.	Na-fumarate	NH_4Cl	H_2O	Yeast	Buffer solut.	Toluol	Days	Temp.	Press.	Fumaric acid or succinic acid	Cu-aspartate	Yield
	g.	g.	c.c.	c.c.(g.)	c.c.	c.c.		°C		g.	g.	%
1	3	6	100	25(1.5)	150	20	7	40	reduce	—	0.14	2.76
2	6	12	150	50(3)	200	0	〃	〃	ordina	—	7.65	72.79
3	0	0	100	25(1.5)	150	20	〃	〃	reduce	—	0	0
4	0	0	150	50(3)	200	0	〃	〃	ordina	—	0	0
5	3	6	100	0	150	20	〃	〃	reduce	—	0	0
6	6	12	150	0	200	0	〃	〃	ordina	—	0	0
7	3	0	100	25(1.5)	150	20	〃	〃	reduce	—	0	0
8	6	0	150	50(3)	200	0	〃	〃	ordina	—	0	0

It was proved that no aspartic acid was synthesized in the absence of yeast or ammonium chloride.

9	3	6	125	25(5.5)	150	20	18	35	reduce	—	2.40	43.4
10	6	12	150	50(11)	200	0	13	37	ordina	—	5.84	53.0
11	3	6	70	50(3)	75	〃	2	〃	〃	—	3.60	65.2
12	〃	〃	〃	25(5)	100	〃	10	35	〃	F. 0.65	0.45	8.1
13	〃	〃	〃	〃	〃	〃	11	〃	〃	〃 0.68	0.70	12.6
14	〃	〃	〃	〃	〃	〃	12	〃	〃	〃 1.55	0.40	7.2
15	〃	〃	〃	〃	〃	〃	13	〃	〃	〃 0.47	3.02	54.2
16	〃	〃	〃	〃	〃	〃	14	〃	〃	〃 0.53	3.05	55.2
17	2	4	40	50(10)	〃	〃	3	〃	〃	〃 1.65	0	0
18	〃	〃	〃	〃	〃	〃	4	〃	〃	〃 1.20	0.70	18.9
19	〃	〃	〃	25(5)	〃	40	3	〃	〃	〃 1.20	0	0
20	〃	〃	〃	〃	〃	〃	5	〃	〃	〃 1.60	0	0
21	〃	〃	〃	〃	〃	〃	9	〃	〃	〃 1.70	0	0

22	〃	〃	〃	30(1.5)	70	0	4	45	〃	S.	0.80	1.30	35.2
23	〃	〃	〃	〃	〃	〃	7	〃	〃	〃	0.90	1.40	37.9
24	〃	〃	〃	〃	〃	〃	11	〃	〃	〃	0.50	1.55	42.2
25	〃	〃	〃	〃	〃	〃	14	〃	〃	〃	0.70	1.80	48.8
26	〃	〃	〃	60(3)	〃	〃	4	〃	〃	〃	0.98	0.30	8.1
27	〃	〃	〃	〃	〃	〃	7	〃	〃	〃	0.80	0.95	25.7
28	〃	〃	〃	〃	〃	〃	11	〃	〃	〃	0.70	1.00	27.1
29	〃	〃	〃	〃	〃	〃	15	〃	〃	〃	1.00	1.21	32.2
30	〃	〃	〃	90(7.5)	〃	〃	4	〃	〃	〃	1.80	0	0
31	〃	〃	〃	〃	〃	〃	7	〃	〃	〃	1.30	0	0
32	〃	〃	〃	〃	〃	〃	10	〃	〃	〃	1.20	0.90	24.4
33	〃	〃	〃	〃	〃	〃	15	〃	〃	〃	1.10	1.00	27.1

Mechanism of synthesis.



$$\text{I.}\ \begin{array}{l}\mathrm{HOOCHC}\\ \ \|\\ \mathrm{CHCOOH}\end{array} + \mathrm{NH_3} \longrightarrow \mathrm{HOOCCH(NH_2)CH_2COOH}$$

$$\text{II.}\ \begin{array}{l}\mathrm{HOOCHC}\\ \ \|\\ \mathrm{CHCOOH}\end{array} \longrightarrow \begin{array}{l}\mathrm{CH_2COOH}\\ \,|\\ \mathrm{CH_2COOH}\end{array} + \mathrm{NH_3} \longrightarrow \begin{array}{l}\mathrm{CH(NH_2)COOH}\\ \,|\\ \mathrm{CH_2COOH}\end{array}$$

$$\text{III.}\ \begin{array}{l}\mathrm{HOOCHC}\\ \ \|\\ \mathrm{CHCOOH}\end{array} \longrightarrow \begin{array}{c}\mathrm{HOOCCH(NH_2)}\\ |\\ \mathrm{CH_2COOH}\end{array} \longrightarrow \begin{array}{l}\mathrm{CH_2COOH}\\ \,|\\ \mathrm{CH_2COOH}\end{array} + \mathrm{NH_3}$$

It seems clear from the result described above that there are present in yeast 2 mechanisms capable of dealing with aspartic acid :

1. a mechanism which the formula I shows, is certified by Nos. 12—21.

2. a mechanism which the formula II or III shows (it is impossible to determine which formula II or III is true by the present experiment), is certified by Nos. 22—33.

SUMMARY.

1. It has been proved that l–aspartic acid is synthesized from fumaric acid and ammonium chloride by yeast and no aspartic acid is synthesized in the absence of yeast or ammonia.

2. The best yield for the present is 75.59% of the theoretical.

3. It is presumed that there are 2 mechanisms in reference to the synthesis of l–aspartic acid :

a) Fumaric acid + NH_3 → l–aspartic acid
b) Fumaric acid + NH_3 → l–aspartic acid → succinic acid + NH_3
Fumaric acid → succinic acid + NH_3 → l–aspartic acid.

ON THE PROBABILITIES OF PROLINE PRECIPITATION BY PHOSPHOTUNGSTIC ACID.

By

E. Takahashi and K. Srirahama.

(Received June 27th ,1928)

When we have treated the hordein of naked barley by Van Slyke's method, the solutions were mixed with phosphotungstic acid and put aside for 23 days at a room temperature, by some accident, instead of 48 hours as stated in the method.

After analysis, we noticed, different from our former investigation and from the results of Hoffman and Gortner, the great increase of non-amino nitrogen in basic fraction and also great decrease of the same nitrogen in the filtrate from bases.

Through calculation refering the data of our precise investigation previously carried out, we found the fair agreement between the increased and decreased quantities of the nitrogen, showing the one transferred from the other. Gortner and Sandstrom have already shown that the high histidine nitrogen value might possibly be caused by proline being precipitated with the bases and appearing in the histidine fraction.

From these results, we may consider that the proline must be almost precipitated when it is stood for a long time as above.

ON THE CHANGE OF ALCOHOL SOLUBLE PROTEINS DURING GROWTH OF MALTS.

By

E. Takahashi and K. Shirahama.

(Received June 27th., 1928)

It is studied on the change of alcohol soluble proteins (Hordein A and B) from naked barley during growth (of 24, 48 and 72 hours in the thermostat after the washing) of malts.

The moisture and ash contents, total nitrogen, various forms of nitrogen and the peptization of proteins are all regularly changed.

The results show that "Bynin" is not a characteristic protein of malts as Osborne said, however not also the same protein with hordein as Kraft and Lüers concluded.

During germination, hordein are greatly decomposed to the water soluble protein substances and at the same time the hordein itself is also changed gradually in its chemical constitution. From these points, the name of "Bynin" is rather corresponded to a general name for the protein which underwent some changes in the course of germination and having properties soluble in alcohol.

STUDIES ON THE ACIDITIES OF CANE MOLASSES WORT.

By

T. TAIRA.

(Received June 29th., 1928)

It is generally recognised that the rate of fermentation and yields of alcohol by living yeast depend on the fermentative power of the yeast employed on one side and the hydrogen ion concentration and buffer capacity of the medium on the other. Notwithstanding the cane molasses is one of the most important raw material for alcohol industry, no investigations refering to the studies of these factors and optimal hydrogen ion concentration for yeast growth are found in literature, probably owing to its dark colour and impracticability for indicator method.

The cane molasses behave weak acid reaction and are used in the distillery of this country without any precaution, but in Hawai, Louisiana, and Cuba it is reported that 1–10 gallons of sulphuric acid are added per 1000 gallons of wort, and also on Formosan molasses R. Nakazawa (Report of Government Institute of Formosa. No. 3, 129, 1913) observed that the better yields of alcohol were obtained by adding lactic and sulphuric acid. W. L. Owen (J. Ind. Eng. Chem., **16** 387, 1924) reported on his valuable work that "The acidification of cane molasses is one of the most important steps preparatory to its convertion into alcohol. The amount of acid required have been found to vary not only with the type of molasses used but also with the race of yeast employed for its fermentation." But his conclusion does not based on the hydrogen ion concentration and he continued that " Apparently, the accurate and rational method for determing the acid requirement of molasses for distillary purpose would be by measuring the hydrogen ion concentration."

Therefore the present author used the electrometric method by an apparatus consisting of type K potentiometer, Weston standard cell and type R galvanometer of Leed and Northrup Co. in conjunction with hydrogen electrode against saturated calomel half cell connected by an agar–KCl–bridge, and estimated the hydrogen ion concentration, buffer capacity and optimal pH value for alcoholic fermentation of the cane molasses wort.

SUMMARY OF THE RESULTS.

1. The hydrogen ion concentration of cane molasses wort has been investigated by gas chain method for distillery purpose. It varies from pH 5.06—5.93 at 20° Bolling.
2. On heating or reservation, the pH diminishes 0.3—0.56.
3. The buffer capacity has been shown by electrometric titration curve.
4. The titrable acidities of cane molasses wort by electrometric method were 18—36 c.c. of 1/10 n NaOH on 100c.c. of wort, and by ordinary indicator method using litmus paper were 20—35c.c. and when used phenolphthalein as an indicator were 36—74c.c. of the same.
5. It is observed that the cane molasses wort ferments with good results on the pH range of 3.5—6.0 and the optimal range is pH 4.0—5.0 by the yeast No. 396 of our laboratory which is the powerful and widely distributed species in this land.

(Government Research Institute, Formosa.)

THE POLAROGRAPHIC STUDIES ON THE FERMENTATION PRODUCTS. PART II.

By

MASUZO SHIKATA and KENJIRO SHOJI.

(Received July 4 th., 1928)

(1) The reduction potentials of α, β, γ–ketonic acids were measured with

the dropping mercury cathode and the polarograph. α–ketonic acid (pyruvic acid) was much more reducible than β–and γ–ketonic acids (acetoacetic acid and laevulinic acid).

In 0.02n LiOH solution, containing 0.1% of ketonic acid, tne reduction potentials at 20°C were as the following:

Pyruvic acid	−1.304V(from In calomel electrode)
Ethylacetoacetate	−1.906V(〃 〃)
Laevulinic acid	−1.981V(〃 〃)

(2) In sake (Japanese rice wine) and Shoyu (Japanese sauce from soya beans), it was hardly possible to conclude that this wave was due to the latter, as the other possibilities were still remaining, for example, caromel prepared by roasting pure glucose giving rise a wave quite similar to that position.

(3) β– and γ–ketonic acids are difficultly determinable by the polarographic method.

(4) Still we might say, that the reduction waves less negative than −1.7 volts were not due to β– or γ–ketonic acid.

RESEARCHES ON THE ELECTROLYTIC REDUCTION POTENTIALS (PART V).

ON THE REDUCTION POTENTIALS OF KETONIC RADICALS AND THEIR RELATION TO THE CHEMICAL CONSTITUTIONS (*CONTINUED*)

By

Masuzo Shikata and Isamu Tachi.

(Received July 4 th., 1928)

Summary.

(1) The reduction potentials of acetylacetone, acetonylacetone, benzoylacetone, dibenzoylmethane and diphenyltriketone were measured with the dropping mercury cathode and the polarograph.

(2) In order to show the relation of reduction potentials of these compounds to their concentrations and hydrogen ion concentrations, the so-called theoretical values of R. P. were calculated by the Nernst formula of

the reversible reduction type assuming two atoms of hydrogen taking part in the reduction, and were compared with the observed values at 25°C

$$\pi = -\frac{0.05911}{2}\log\frac{k'}{[H^{\cdot}]\,[\mathit{Reducible\ compound}]}$$

EXPERIMENTS.

(3) Results of one part of the experiments were given in Fig. 5 (in original paper) showing P_H reduction potential curves.

(4) For acetylacetone, taking the reduction potential (–1.097 V) of $1.033\cdot 10^{-2}$ molar solution in the mixture as a basis of a calculation we found

$$\log k' = 31.09$$

For acetonylacetone

$$\log k' = 32.94$$

(–1.152 V for 1.0 10^{-3} molar solution in the mixture [0.01 n HCl 0.1 n KCl])

For benzoylacetone

$$\log k' = 22.70$$

(–0.901 V for 1.0 10^{-3} molar solution in the mixture [0.01 n HCl 0.1 n KCl])

For benzoylmethane

$$\log k' = 22.90$$

(–0.887 V for 1.0 10^{-3} molar solution of 50% alcoholic medium [0.01 n HCl 0.1 n KCl])

For diphenyltriketone,

$$\log k' = 2.46$$

(–0.311 V for 1.0 10^{-3} molar 10% alcoholic solution of 0.1 n NH_4Cl)

(5) 1—3 diketone, such as acetylacetone, benzoylacetone, dibenzoylmethane showed two distinct reduction potentials, due to enol– and ketoforms of these compounds. Further their enol forms were always more reducible than their ketoform.

(6) The negatifying effect of ethyl alcohol to the reduction potential of benzoylacetone was tested with the result that their R. P. changes linearly with the alcoholic concentration.

(7) The solubility of benzoylacetone to water was calculated to be 2.36×10^{-3} mol per liter at 25°C.

(8) The group and orientation effects on the reduction potentials of ketonic compounds were found as the following.

1, 2 diketones were more reducible than 1,3 diketones, for example:

Diacetyl and acetylacetone	0.802 V
Benzil and dibenzoylmethane	0.616 V

1, 3 diketone was more reducible than 1, 4 diketone, although the difference was much less conspicuous than the former cases.

Acetylacetone and acetonylacetone	0.055 V

Substitution of methyl group with phenyl group made R. P. more positive as mentioned in Part IV of our papers.

Benzoylacetone and acetylacetone	0.264 V
Dibenzoylmethane and acetylacetone	0.385 V

With regard to 1, 2 diketone and 1, 2, 3, triketone, contrary to our expectation, the former was more reducible than the latter, for example.

Benzil was more reducible than diphenyltriketone. This is much noticeable as diketones were far more reducible than monoketone as was reported in our previous paper.

RESEARCHES ON THE ELECTROLYTIC REDUCTION POTENTIALS. (PART VI.)

ON THE REDUCTION POTENTIALS OF NITPOPHENOLS.

By

Masuzo Shikata and Mamoru Watanabe.

(Received August 20th., 1928)

Summary.

(1) The electrolytic reduction potentials of ortho–, meta–, and para-nitrophenol were measured with the dropping mercury cathode and the polarograph.

(2) Analogous in the case of nitrobenzene [Shikata: Trans. Faraday Soc., No. 61 XXI 42 (1925)] the first stage of reduction was thought to be the system nitrophenol-nitrosophenol.

(3) Two waves of reductions were found in slightly alkaline solution, in which the easily reducible wave was proved to be due to the reduction of the undissociated nitrophenol and the second wave to be that of the ionised nitrophenol. It should be noticed that negatively charged nitrophenol ion was more difficultly reducible than the undissociated molecule, and this was quite contrary to the case of pyridine, that pyridine ion (cathion) was more easily reducible than the undissociated pyridine molecule at the dropping mercury cathode.

(4) Reduction potentials of these compounds in the solutions of various hydrogen ion concentrations were compared with the calculated values from the following formula at 25°C, using the 1n calomel electrode as the standard electrode.

$$\pi = -\frac{0.05911}{2}\log\frac{k'}{[H^{\cdot}]^2[\text{Reducible compound}]}$$

(5) For o-nitrophenol, taking the reduction potential (0.072 V) of 10^{-3} molar solution in the 0.1n HCl containing 3% ethanol as the standard,

we found $\log k' = -2.57$

and for ionised o-nitrophenol,

$\log k'' = 0.02.$

For m-nitrophenol, by taking the reduction potential (0.043 V) of 10^{-2} molar solution in 0.1n HCl as the standard, we found

$\log k' = -2.55$

and for ionised m-nitrophenol,

$\log k'' = 3.46.$

For p-nitrophenol, i. e. 0.090 V under the same conditions as the former standard, we found

$\log k' = -0.95$

for the reduction of free molecule and

$\log k'' = 5.51$

for that of p-nitrophenol ion.

(6) Concordance of the observed values with the calculated values was tolerably good in the slightly acidic solutions but the former was much more positive than the calculated value in the alkaline solution for all nitrophenols.

(7) The following series may be given for the reducibilities of isomeric nitrophenols and nitrobenzene.

o-nitrophenol > m-nitrophenol > nitrobenzene > p-nitrophenol.

(College of Agriculture, Kyoto Imperial University)

BIOCHEMISCHE UNTERSUCHUNG ÜBER FIXATION DES FREIEN STICKSTOFFS DURCH AZOTOBACTER.

I. MITTEILUNG: ÜBER DAS AUSSCHEIDUNGSVERMÖGEN DER N-HALTIGEN VERBINDUNGEN DURCH LEBENDEN AZOTOBACTER.

von

Dr. HACHIRO KUMAGAWA und KOTARO OKAMOTO.

(Ausgegangen am 4, August 1928)

Dass der im Boden frei lebende Azotobacter den atomosphärischen Stick-

stoff fixiert und damit eigenen Bakterienkörper sich aufbaut, der nach dem Tod des Azotobacters durch Abspaltung des Stickstoffs die für höhere Pflanzen aufnahmswürdigen N–Verbindungen bildet, ist längst bekannt. Aber haben wir keine Versuche über die Frage, ob der lebende Azotobacter bei Stoffwechsel die N-haltigen Verbindungen ausserhalb des Bakterienkörpers ausscheide. Diese Frage ist sehr wichtig nicht bloss für Azotobacter, sondern für den Stoffwechselvorgang sonstigen Backterien, besonders für die Naturbestimmung des Bakterientoxins-und Farbstoffs.

Für die Untersuchung benutzten wir zwei arten des Azotobacters, Azotobacter Chrooccocum und Az. Vinelandii, und als Nährboden das Ashby'sche Agarmedia (modifiziert Gulukose für Mannit). Das Nährmedia wurde in Petrischen Schale eine Platte gemacht, die durch ihren Durchmesser möglichst genau 3 gleichgrosse Partien (A, B, und C) abgetrennt. Eine Platinöse des Azotobacters wurde in obere Partie (A genannt) geimpft mit Drigarsky-Conradiischem Stab, und in Brutofen auf 28°C zusammen mit den nicht geimpften Kontrollschalen inkubiert. Nach bestimmtem Kulturtage die geimpfte Partie (A), nicht geimpfte Partie (B und C) bei Impfschale und gesammtmenge Agar bei nicht geimpften Schalen wurde ihr N–Gehalt durch Folin-Farmersche Methode kolorimetrisch bestimmt.

Nach unsere Resultate 1). N–Zunahme am 4. und 7. Kulturtage bei nicht geimpften Partie (B und C) gering nachweissbar. 2). Am 14. und 21. Kulturtage bei nicht geimpften Partie (B und C) mehr deutlicher N–Zunahme und geringe N–Abnahme bei Impfpartie (A). Diese Erscheinung spricht folgende Tatsache: 1). Der lebende Azotobacter scheidet N-haltige Verbindungen ausserhalb seines Körpers. 2). Die autolytische N–Verbindungen wurden ausser dem Bakterien-Körper ausgeschieden.

Nächstens experimentierten wir über die Natur der ausserhalb des Bakterienkörpers ausgeschiedenen N–haltigen Verbindungen. Möglichst genau abgemessenen 40 c.c. Nährboden wurde in Petrischer Schale eine Platte gemacht, die durch ihren Durchmesser 2 Partien (A und B) abgetrennt, und in Partie A eine Platinöse Bakterien geimpft. Nach bestimmtem Kulturtage (Kulturtemperatur 28–30°C) nahm Impfpartien (A) und nicht-geimpften Partien (B) aus 5 Impfschalen in Erlenmeyerschem Kolben (300 c.c. Kapacität) auf (je Kolben enthält 100 c.c. Nährboden). Die 2 oder 3 Kontrolschalen (der gesammt 100 c.c. Nächrboden enthält) wurden gesammt in einen Erlenmeyerschen Kolben auf genommen. Für Auslösen von Agar, addierte man je 10 c.c. 2.5% HCl in je eine Kolben und kochte in 100°C Wasserdampfbad um 15 Minuten, dann in Zimmertemperatur gelassen und goss in 100 c.c. Messkolben quantitativ, erfüllt mit Wasser bis Marke. Aus diesem Material bestimmten wir Amino–N mit 40 c.c. durch Formol-Titration nach Sörensen und NH_3–N mit 40 c.c. durch Folin-MacCullumsche kolorimetrische Methode und Gesammt-N mit 10 c.c.

durch Folin-Farmersche.

Nach unsere Resultate war an den Beginn der Kultur das Gehalt des Amino-N gross und des NH_3–N gering an Impfstelle (A) und Kein Amino-N und merkliche Menge des NH_3–N an nicht geimpfte Partie (B), der immer gross geworden. Bakterien-N wurde immerhalb 2 Woche der Kultur Maximum und nahm sich mit Abnehmen der N–Fixation ab. In diesem Falle bildet es sich zuerst NH_3, dann Aminosäure aus NH_3 und zuletzt Bakterienkörpers aus Aminosaüre.

In nicht geimpften Partie (B) nehmen die Menge der ausgeschiedenen NH_3–N immer bis zum letzten Kulturtage, und auch Aminosäure abgescheidet, aber ihre Menge sehr gering.

Durch oben genannten Versuche warnahmen wir sich, 'dass der lebende Azotobacter den N–haltigen Verbindungen ausser seinem Körper ausscheide, die in Form von NH_3 und geringe Menge Aminosaüre. Diese Verbindungen sind aufnehmbar für höhere Pflanzen und wirken als Düngemittel.

BIOCHEMISCHE UNTERSUCHUNG ÜBER FIXATION DES FREIEN STICKSTOFFS DURCH AZOTOBACTER.

II. MITTIEILUNG: ÜBER DAS VORGANG DES N–FIXATION DURCH AZOTOBACTER.

von

Dr. HACHIRO KUMAGAWA und KOTARO OKAMOTO.

(Ausgegangen am 4, August 1928)

Betreffend des Vorgangs der N–Fixation durch Azotobacter wurde bisher vielfach bestritten. Wir betrachteten nach unsere Arbeit (I. Mitteilung), dass das nascierende Wasserstoff, der durch Spaltung des Kohlehydrates im Nährboden bei Stoffwechselvorgang gebildet wird, erst mit atmosphärischem N sich verbinde und NH_3 bilde. Der Nachweiss von HNO_3, HNO_2, bei flüssigen Kultur (Aschby's Media) des Azotobacters ist immer negativ und NH_3 fast Konstant possitiv. Neuerdings wies Kostyschew und seine Mitarbeitern grösse menge Ammoniak und Aminosaüre in dem Filtrate der flüssigen Kultur von Az. agilis und schloss, dass die erste Produkte der N–Fixation NH_3 sein kann.

Wir lasen Kostyschew'sche Veröffentlichung in dem Laufe unseren Ver-

suche, doch Kostyschew'sche Arbeit war Experimentalfälle wenig und nicht systematisch.

Unsere Untersuchungsmethode ist anders als seine Methode. Als Nährboden benützten wir einfache Fest-Media, das 1.5% Glukose, 0.5% einbasische Kaliumphosphat enthält in 2% Agar. Dieses 140 c.c. Nährmedia in 500 c.c. Erlenmeyer-Kolben gegossen, eine platinöse Bakterien (Az. chr. und Az. vinelandii) geimpft mit Dorigarsky-Konradii'schem Stab, in 28°C incubiert zusammen mit die nicht-geimpfte Kontrolkolben.

Nach bestimmtem Kulturtage, addierten wir je 10 c.c. 2.5% HCl in je eine Kolben, kochten in 100°C Strombad um 20 Mimuten für Auslösen des Agar, in Zimmertemperatur gelassen, goss quantitativ 150 c.c. Messkolben und erfülten mit Wasser bis Marke. Nach gutem Ausschütteln, bestimmten wir NH_3-N in 60 c.c., Gesamt N in 10 c.c. und Amino-N in 60 c.c. aus diesem Material.

Resultate : Freier N wird anfangs als Ammoniak fixiert durch Bakterien, ist aber seine Menge sehr gering, und am 4. bis 7. Kulturtage deutliche Zunahme des NH_3, und spätere Abnahlme. Aminosaüre nimmt immer in ganzen Kulturdauer zu. Es ist klar, dass NH_3 zu Aminosäure sich ändert. In diesem Periode nehmen der sonstigen N. d.h. der Backterin-N immer zu, es beruht auf der Veränderung der Aminosaüre zu Bakterien-N.

In späterem Kulturtag (28. Tag) nahm NH_3 deutlich zu, und Bakterin-N deutlich ab. Es zeigt dass, der Bakterien-N zu Ammoniak sich abspaltet.

Nach oben beschriebenen Resultate ist es klar, dass der Ammoniak der erste Produkt der Fixation des freien N sein soll, und aus diesem zu Aminosaüre und spalter zu Bakterienkörper aus Aminosüre bildet. Bei Autolyse der Bakterien spaltet Bakterien N sich erst zu Aminosaüre und dann Ammoniak.

ON THE ERGOSTERIN ISOLATED FROM JAPANESE MUSHROOM *CORTINELLUS SHIITAKE.*

By

Mizuho Sumi.

(Received September 8th., 1928)

The author had previously suceeded in isolating ergosterin ($C_{27}H_{42}O$) from Japanese mushroom, *cortinellus shiitake,* and consequently, he undertook to study on its antirachitic property. The results obtained thereby may be summarized as follows: —

(1) Ergosterin irradiated by ultra-violet rays possesses, in such a minute quantity as 0.0001 mg., the healing activity towards a rat suffering from rachitis, thirty minutes being sufficient for the irradiation.

(2) From the consideration on the relation between the chemical constitution of ergosterin and its antirachitic potency, the alcohol group held therein seems to play no rôle. This is evident from the fact that the activity is presented by irradiating several sorts of esters. Again, the double bond held by ergosterin is found to bear great importance, as is evidenced by the fact that it loses its potency by being saturated with hydrogen, or again its isomer, isoergosterin, prepared by simply changing the position of the double bond loses the same property even on irradiation.

(3) Again, by dosing a rat with non-irradiated ergosterin and then irradiating the rat by ultra-violet rays, healing activity is also presented, i.e., the ergosterin in the rat's body responds to the ultra-violet rays, giving rise to the same effect.

(4) By examining the absorption band of the spectrum by ergosterin there is a new band at $250\mu\mu$, besides those reported by many authorities, viz., those at $293\mu\mu$, $280\mu\mu$, $270\mu\mu$, and $260\mu\mu$. The result of the animal experiment shows clearly the relation between these five absorptions and antirachitic activity.

ON THE COMPONENTS OF THE ROOTS AND RHIZOMES OF SOME WILD UNCULTIVATED PLANTS USED FOR FOOD IN CHOSEN.

By

Fujio Narita.

(*From the labolatory of the Biochemical Department of the Keijo Imperial University, Prof.* G. Sato.)

(Received Septembar 14th., 1928)

Roots and Rhizomes of many wild uncultivated plants are used in Chosen as the food in spring and autumn, especially after a bad harvest. I took some chemical examinations on several kinds of them.

a) Root of KIKYO (Chinese bell flower; Platycodon grandiflorus, DC.)

b) Rhizome of HIMEIZUI (a kind of a common Solomon-seal; Polygonatum humile, Fischer.)

c) Root of HIRUGAO (larger bind flower; Calystegia sepium, R. Br.)

d) Root of NAZUNA (Pickpurse; Capsella Bursa. v. pastrys L.).

e) Root of NIGANA (Lactuca Thumergiana, Max.).

The general components were examined by usual chemical methods. Calculating in the dry matter, the results are as follows:

	KIKYO %	HIMEIZUI %	HIRUGAO %	NAZUNA %	NIGANA %
Grude protein	2.44	9.34	20.17	31.54	9.65
Portein	1.59	4.22	9.91	13.66	5.34
Crude fat	1.16	1.23	3.78	1.54	2.38
Crude ash	2.30	5.20	8.28	6.72	5.49
Crude fiber	9.06	15.57	10.65	11.76	9.28
N-free extract	85.04	68.66	57.11	48.45	73.20

Secondary the samples are extracted with absolute ethyl alcohol, methyl alcohol and boiling water, and finally digested with 3% HCl. The forms and amounts of carbohydrate were determind in each fraction. They are summarised in the following tables:

Reducing and non-reducing sugars (percentage for total sugar).

Samples / Fractions		KIKYO %	HIMEIZUI %	HIRUGAO %	NAZUNA %	NIGANA %
Ethyl alcohol	(1)	10.30	6.35	32.99	28.45	21.17
Methyl alcohol	(2)	5.65	8.78	10.60	15.99	24.42
(1) plus (2)		15.95	15.13	43.59	44.44	45.95
Hot water	(3)	60.38	57.52	12.36	2.86	34.22
3% HCl	(4)	23.93	27.35	44.05	68.89	20.18

Reducing sugar (percentage for total reducing sugar).

Samples / Fractions		KIKYO %	HIMEIZUI %	HIRUGAO %	NAZUNA %	NIGANA %
Ethyl alcohol	(1)	74.04	33.10	81.54	68.07	61.42
Methyl alcohol	(2)	11.82	26.46	6.81	31.93	20.01
(1) plus (2)		85.86	59.56	68.35	100.00	81.43
Hot water	(3)	14.14	40.44	31.65	0.00	18.57

Non-reducing sugar (percentage for total non-reducing sugar).

Samples / Fractions		KIKYO %	HIMEIZUI %	HIRUGAO %	NAZUNA %	NIGANA %
Ethyl alcohol	(1)	0.76	1.41	9.42	16.29	15.44
Methyl alcohol	(2)	4.76	5.51	12.44	11.09	25.05
(1) plus (2)		5.52	6.92	21.86	27.38	40.49
Hot water	(3)	67.08	60.68	12.68	3.73	36.45
3% HCl	(4)	27.40	32.40	65.46	68.89	23.05

The ratio of ketose to aldose (Ketose/Aldose).

Samples / Fractions	KIKYO	HIMEIZUI	HIRUGAO	NAZUNA	NIGANA
	%	%	%	%	%
Ethyl alcohol (1)	1.79	2.64	1.98	2.94	2.84
Methyl alcohol (2)	9.37	5.39	2.45	5.94	2.75
Hot water (3)	58.70	0.20	0.32	0.65	8.84
3% HCl (4)	0.46	0.27	0.38	0.68	0.33
Total quantity	3.64	0.38	0.80	1.34	2.26

The reducing and non-reducing sugars were estimated, directly or after hydrolysis, by Bertrand's method. For the estimation of ketose Campbell-Hanna's procedure was followed with some modifications.

The principal forms of carbohydrate are : inulin (KIKYO, NIGANA), starch (HIRUGAO, NAZUNA) and mucilage (HIMEIZUI).

Inulin was prepared from the KIKYO-root by ordinary procedure. Investigations of optical rotation and estimation of sugar (both Bertrand's and Campbell-Hanna's methods) showed the extra purity of inulin.

Mucilage was also prepared from the hot water extract of HIMEIZUI-rhizome by precipitating with alcohol. Large amount of mannose was found as its phenylhydrazone from the acid hydrolysate.

Note :– Modifications on Campbell-Hanna's methods.

1). The blue coloured content of heated tube was diluted with water five times of its volume before the addition of sulphuric acid, the titration with n/200 $KMnO_4$ was then carried out.

2). As aldoses produces the blue colour, even if the intencity is one-twentyth as great as fructose, the following formula of calculation was adopted for estimating fructose mixed with aldose :

K'……Fructose value obtained by Campbell-Hanna's method.
K ……True fructose value.
Z ……Total sugar value by Bertrand's method.

$$K' + \frac{1}{20}(Z-K) = K'$$

$$K = \frac{K'-0.05}{0.95}$$

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A MICRO pH ELECTRODE.

By

ARAO ITANO.

(Received October 7th., 1928)

The description of an electrode, A Type, by which a determination of hydrogen ion concentration in a minute quantity of sample viz. 0.02 c.c. or less can be carried out satisfactorily.

The electrode is made of a simple capillary tube, less than 1 mm. in its diameter, of which both ends are enlarged so that a platinum wire electrode and a sharply pointed agar bridge are inserted, one in each opening, and connected up in a chain as usual.

This type is used only with quinhydrone while B Type which is in course of construction at present can be used with the hydrogen gass electrode under special precautions such as CO_2 tention, oxidation etc.

INVESTIGATION ON A SATURATED CALOMEL ELECTRODE.

By

ARAO ITANO.

(Received October 7th., 1928)

This paper deals with a comparative study of two types of the calomel electrode of the following description, and also with an equation by which pH value is obtained from the reading on the millivoltmeter with the saturated calomel elctrode, using Table for pH for N/10 calomel electrode.

Two types of electrode used were as follows:

I. N/10 KCl calomel electrode which was prepared very carefully according to the standard method.

II. Saturated KCl calomel electrode which was prepared as follows:

Six grams of pure calomel, 15 c.c. purified mercury and 50 c.c. saturated KCl solution were mixed together and shaken vigorously in absence of air, for 5 minutes, and in a vessel.

Table I.

Comparative Results of N/10 and saturated Calomel Electrode.

Temp. °C	Known pH	Kind of Calomel Electrode.	Millivolts.	pH* Determined.
28.	7.26	I.	−0.054	7.25
〃	〃	II.	+0.036**	7.27

* an average of several determinations by three different experimentors.

** From 0.036 m.v. which was read on the millivoltmeter, pH was calculated according to the following equation :

$$\lceil (x \times \text{T.F.}) - 0.0874 = \pi \cdots\cdots\cdots\cdots\cdots\cdots \text{(A.)}$$

where

x :– reading on the millivoltmeter.

T. F :– temperature factor.

0.0874,-: difference of potential between N/10 and saturated calomel electrode at 18°C.

π :– E. M. F. noted in Table for pH Values etc. (Itano: loc. cit. p. 206)

In this case, therefore

$$(0.036 \times 0.966) - 0.0874 = -0.0526 \text{ or pH } 7.27 \rfloor$$

II electrode has the following merits :

(1) Ease in its preparation.

(2) No change in its potential by shaking or by evaporation.

(3) Better electric conductivity is offered owing to the high concentration.

The potential of II calomel electrode at 18°C was calculated and was found to be 0.2514.

ON THE OXIDATION OF "BIOSTERIN" BY OZONE.

By

ZIRO NAKAMIYA.

(Received October 29th., 1928)

The author prepared biosterin ozonide by saturating the chloroform solution of biosterin with ozonized oxygen under continuous cooling and from its solubility towards petroleum ether, separated it into two fractions, viz. the soluble (mono-ozonide ?) and the insoluble ones (di-ozonide ?). Each fraction gave similar decomposition products when it was boiled with water thus hydrogen peroxide, carbonic acid and acetaldehyde were detected among the generated gases, the last named substance being converted into dimedon compound. The resulted aqueous solution was subjected to steam distillation and the distillate was treated with ether, whereby an almost colorless, acidic oil, having the properties resembling in many respects to a derivative of sesquiterpenalcohol, was obtained from the etherial extract. Further studies on this substance will be carried out later on. From the aqueous solution of the above distillate were isolated, formic acid, formaldehyde and butyladehyde. The residue of steam distillation was again treated with ether and from this etherial solution two reddish brown viscous masses were separated, the one had neutral reaction, with the average composition, C 67–72%, H 9–10% ; while the other was acidic in nature and had the composition, C 63–65%, H8–9%. These two substances were largest in amount among the decomposition product found. They are most probably formed from the primary decomposition products by polymerization during the operation.

After treatment with ether, from the final residue, acetic acid, formic acid, formaldehyde and diacetyl were isolated, the last one being identified as its osazone as well its semicarbozone.

ON VITAMINS IN SAKE-CAKE.

I. ON BITAMIN B IN SAKE-CAKE.

By

E. Takahashi and H. Lim.

(Received November 25th., 1928)

The present paper reports the results of the investigation upon Vitamin B in sake-cake. The experiments were carried out with chickens and albino rats and the presence of the B factor was fully proved. These animals were fed with the dried sake-cake or the concentrated solution which was prepared by extraction of the cake with alcohol and refining with phosphotungstic acid as usual method.

It has been reported by many authors that the two factors, antineuritic and growth promoting, may be detected in so-called Vitamin B. Our investigation made clear also the presence of both these factors in the cake. The diets of the various lots as well as its results obtained by chickens were as following tables.

I. Diets of the Various Lots.

Lot no.	1	2	3	4	5	6	7	8
Sake-cake	30%	50%	%	%	%	%* (1c.c.)	%* (3c.c.)	%
Ground Yellow Corn			10		30			
Brewers'Yeast			20	30		20		
Polished rice	39	19	39	39	39	39	39	69
Meat residue	20	20	20	20	20	20	20	20
Fine oyster shell	4	4	4	4	4	4	4	4
Bone meal	4	4	4	4	4	4	4	4
McCollums' salts (185)	1	1	1	1	1	1	1	1
Cod liver oil	2	2	2	2	2	2	2	2

* Vitamin B concentrated solution. One cubic centimeter of this solution is corresponded to ten grams of Sake-cake.

II. The Results of Experiments with Chickens.

Lot no.	Number of chicken	Vitamin supplement			No. of chicken suffered polyneuritis	Average wt. of chicken at 10 weeks.
		Sake-cake	Corn	Yeast		
		%	%	%		g.
1	4	30			0	626
2	2	50			0	all dead.
3	3		20	10	0	782
4	3		30		0	285
5	3			30	0	694
6	3	(1 c c.)	20		0	420
7	3	(3 c.c.)			0	313
8	2				2	all dead.

The diets and its results of experiments with rats were as follows.

III. The Results of experiments with rats.

No. of animal	Body weight		duration of experiment.	Diets
	initial wt.	final wt.		
	g.	g.	days.	
No. 65 ♂	50	30	38*	Basal diet alone.
No. 66 ♂	60	30	32*	〃 〃 〃
No. 69 ♀	45	150	77	Basal diet+0.5 c.c. of Vitamin B conc. sol. per day.
No. 72 ♂	70	165	77	〃 〃 +〃 〃 〃 〃 〃 〃 〃 〃
No. 73 ♂	60	140	77	〃 〃 +〃 〃 〃 〃 〃 〃 〃 〃
No. 75 ♂	20	180	77	Basal diet+1 g. of Sake-cake per day.
No. 78 ♀	30	170	77	〃 〃 + 〃 〃 〃 〃 〃
No. 80 ♀	30	325	77	〃 〃 +1 g. of brewers'Yeast per day.
No. 81 ♂	20	335	77	〃 〃 + 〃 〃 〃 〃 〃 〃

* dead.

As the origin of B factor in the cake we must consider upon the Sake-yeast which is mixed with the rice residue, but the comparative study with beer-yeast regarding its not quantity has not yet been made.

ON VITAMINS IN SAKE-CAKE.

II. ON VITAMIN C IN SAKE-CAKE.

By

E. TAKAHASHI and H. LIM.

(Received November 25th., 1928)

In the former paper, we have already reported on the presence of vitamin B in Sake-cake. Further experiments showed the presence of antiscorbutically active factor in the cake, upon which will here be informed

Quinea pigs were used for the experiment and as basal ration Oat, wheat bran and cow's milk autoclaved for one hour at 120°C, were fed. As the animals suffered from scurvy in two weeks, cake extract was given and autopsy was made at times.

The extract used as the sample through experiments was the alcohol, water or ether-acetate extracts of the cake, after refined with neutral lead acetate, precipitated with basic lead acetate. The precipitate was decomposed with hydrogen sulphide and condensed. One cubic centimentre of the solution corresponded to the 20 grams of the dried.

The result of experiments was as following table:

The symptoms of scurvy such as hemorrhage, fragility of bone or loosness of teeth are indicated by +, ++, +++, according to advanced degrees; no such symptoms indicated by —.

(A)…………Refined solution, extracted by ethylalcohol.
(B)………… 〃 〃 〃 〃 water.
(C)………… 〃 〃 〃 〃 ether-acetate.
(D)…………Above mentioned A solution, but oxidized passing air through.

Protocols of experimental animal on Basal diet alone or with Antiscorbutic.

Animal number	Quantities of concentrated solution given to animal	Body weight				Duration of experiment	Autopsy Finding							
							Bony system				Hemorrhage			
		Initial	maximum	At a forced feeding day	Final		Shinbone	Teeth	Ribs	Joints	Ribs	Intestines	Joints	Muscles
		wt. g.	wt. g.	wt. g.	wt. g.	days								
10♂	0.0	300	300		260	11*	−	?	+	++	+	−	+	−
12♀	0.0	270	270		210	*11	−	?	++	++	?	−	+	?
5♂	0.0	400	425		260	20	+++	+++	+++	++	++	+++	++	+++
6♂	0.0	340	365		240	20	+++	+++	++	++	+++	++	++	++
7♀	0.0	380	400		235	23	+++	+++	+++	+++	+++	+++	+++	++
20♀	(Radish juice) 3.0	320	400	260	400	*70	−	−	?	−	+	−	+	?
27♀	(Sake-cake) 2.0	350	355	355	190	25	+++	++	+++	++	+++	++	++	++
28♀	(Sake-cake) 2.0	320	320	310	140	23	+++	++	++	+++	+++	++	+++	++
67♂	(A) 2.0	490	490	460	415	50	+	−	++	++	++	−	++	+++
68♂	(A) 3.0	460	490	435	450	*70	−	−	+	+	+	−	+	−
69♂	(A) 3.0	470	505	440	505	*72	−	?	−	++	+	−	−	−
35♀	(B) 3.0	440	440	335	310	46	+	++	++	++	+++	+	++	+
39♂	(B) 3.0	310	310	275	200	40	+	+	+++	+	+++	++	+++	+
45♀	(C) 3.0	415	430	410	330	*27	−	+	+++	++	++	−	+	+
40♂	(C) 3.0	410	410	380	360	33	+	+	+++	+++	++	−	+	−
18♂	(D) 3.0	495	515	480	270	23	++	++	+++	+++	++	++	+++	++
19♂	(D) 3.0	475	520	510	240	30	+++	++	+++	+++	+++	+++	+++	++

* These animals were killed for autopsy ; the other died of scurvy.

STUDIES ON THE ACIDS FORMED BY RHIZOPUS SPECIES. PART VIII.

THE FORMATION OF ACETIC, FORMIC, FUMARIC ACIDS AND ETHYL ALCOHOL FROM PYRUVIC ACID.

By

TEIZO TAKAHASHI and TOSHINOBU ASAI.

(Received November 28th., 1928)

In a former paper[1)], one of us (T. T.) with co-laborater K. Sakaguchi, have called attention as to the occurrence of pyruvic acid in the fluid, which contained calcium carbonate beside all nutrients necessary for the fungus growth, by Rhizopus G. 34 Yamazaki. In the same was added another fact, that this fungus formed lactic acid and ethyl alcohol from fumaric acid, suggesting that ethyl alcohol may, most probably, comes forth from fumaric acid through lactic and pyruvic acid step by step.

Later[2)], the production of ethyl alcohol from acetic acid by the dead cells—acetone fungus—of the same mould was verified. The verification of the excretion of carboxylase[3)] by the same fungus, substantiated the assumption of the formation of ethyl alcohol from fumaric acid *via* pyruvic acid.

Here the cultures of Rhiz. G. 34 and 36 were conducted in the special nutrient medium containing pyruvic acid as the sole source of carbon, for the purpose of ascertaining the behavior of these fungi toward this acid viz. from quite a different point of view as formerly hase been demonstrated (*l. c.*)

The result was that, this fungus produces not only alcohol but also formic, acetic and fumaric acids from pyruvic acid ; affording us one reasonable cause of the shortness of the formation of ethyle alcohol in saccharine medium ; viz. pyruvic acid undergoes at least four defferent ways in its fate of decomposition.

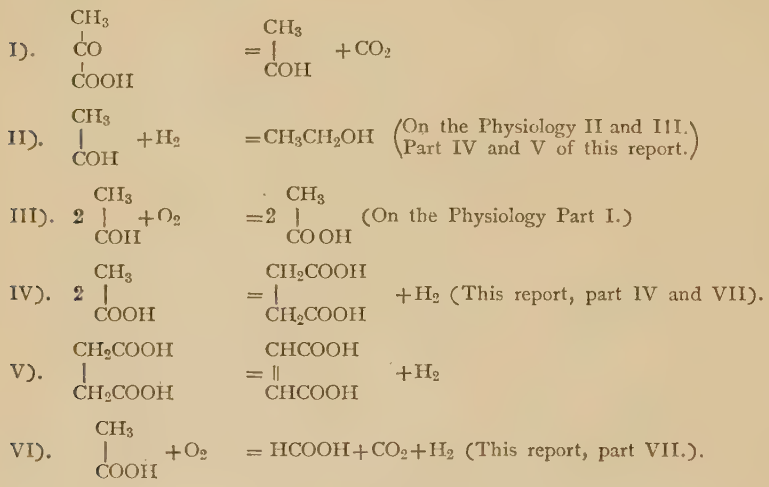
The mechanism of the formation of formic—, acetic—, and fumaric acids by this fungus, in the most possible way, is to be understood, if referred to the former paper[4)], which concerns to the synthesis of fumaric acid by linking two molecules of acetic acid and degradation of the letter into formic acid.

On the related problem, the contradiction of F. Ehrlich and I. Bender's results[6)] to Gottschalk's work[5)], which concerns to the synthesis of furmaric acid from pyruvic acid by Rh. nigricans, must not be over-looked ; because we have experienced altogether in agreement to the result of the latter author's, although our species of the fungus used throughout the experiment was different from foreign authors.

The results of the analyses are shown in the table below :—

Species of Rhizopus.	Dags of culture	Growth of fungus.	Alcohol formed v. %	Acetic acid formed g.	Formic acid formed g.	Fumaric acid formed g.	Pyruvic acid, undecomposed. g.	
							normal	para-form.
G. 34	56	0.2768	0.08	0.1003	0.0537	0.0405	0.1536	0.3160
G. 34	60	0.3011	0.074	0.1292	0.0425	0.0617	0.0960	0.2850
G. 36	58	0.2380	trace (0.05)	0.2489	0.1206	trace	0.1076	0.4002
G. 36	62	0.2542	present (Jodoform test)	0.1262	0.1326	0.0291	0.1113	0.2982

The mechanism of the formation of these acids as well as alcohol from acetic acid may most probably be represented, referring to the former papers, as follows :—



I). $\begin{matrix}CH_3\\ |\\ CO\\ |\\ COOH\end{matrix} = \begin{matrix}CH_3\\ |\\ COH\end{matrix} + CO_2$

II). $\begin{matrix}CH_3\\ |\\ COH\end{matrix} + H_2 = CH_3CH_2OH$ (On the Physiology II and III. Part IV and V of this report.)

III). $2\begin{matrix}CH_3\\ |\\ COH\end{matrix} + O_2 = 2\begin{matrix}CH_3\\ |\\ COOH\end{matrix}$ (On the Physiology Part I.)

IV). $2\begin{matrix}CH_3\\ |\\ COOH\end{matrix} = \begin{matrix}CH_2COOH\\ |\\ CH_2COOH\end{matrix} + H_2$ (This report, part IV and VII).

V). $\begin{matrix}CH_2COOH\\ |\\ CH_2COOH\end{matrix} = \begin{matrix}CHCOOH\\ \|\\ CHCOOH\end{matrix} + H_2$

VI). $\begin{matrix}CH_3\\ |\\ COOH\end{matrix} + O_2 = HCOOH + CO_2 + H_2$ (This report, part VII.).

Experimental.

The species of Rhizopus used : Rhizopus G. 34 and G. 36.

The nutrient medium consisted of 200 c.c. water, pyruvic acid$^{7)}$ 4.5 g, peptone 0.6 g., K_2HPO_4 and KH_2PO_4 each 0.03 g., $MgSO_4$ and $CaCl_2$ 0.02 g. each, NaCl and Fe_2Cl_6 trace, $CaCO_3$ 2.6 g. where by, $CaCO_3$ in the medium, was added after the sterilisation of the solution.

The cultures were incubated at 25–30°C.

The isolation of the products.

The culture solution, freed from the growth of fungus, was neutralised by dilute sulphuric acid, prior to its distillation. The distillate gave any evidence of the presence of aldehyde, provided Schiff's decolourised fuchsin solution was used for the purpose; while the criystals of iodoform decidedly made its occurrence when the solution was warmed in presence of iodine and alkali.

Detection and estimation of acids.

Volatile acids.

The residue of the above distillate was acidified, more or less strong with sulphuric acid, adding tropäolin O O as an indicator.

In the distillate of this acidified fluid, formic acid was detected with ammoniacal Ag–nitrate or aqeous mercuric chloride solution and determined quantitatively after Porters-Ruyssen's method[8] as given in the part VI.

Calcium salts prepared from the acidic distillate gave aceton when the former subjected to the dry distillation, indicating the decided association of acetate in salts.

The acid was determined by titrating the distillate, which was freed, prior to the titration, from formic acid by treating the mixture of both acids with a solution of potassium bichromate associated with sulphuric acid.

Non–volatile acids.

Fumaric acid:

From the ether extract of the residue of the distillate, there comes forth *inter alia* the characteristic crystals of fumaric acid, which gave positive colour reactions to resorcine and[9] β–naphthol[10] in presence of sulphuric acid. It gave insoluble salt by mercuric acetate and reddish brown precipitate by ferric chloride. It melted at 284°C (uncorrected) in a sealed tube.

p–Pyruric acid:

The filtrate from the crystals of fumaric acid, was neutralised by barium carbonate and after concentrating to almost dryness was treated by 80% alcohol, which dissolves barium lactate from the mixture of the salts.

The soluble part viz. the filtrate gave no decisive reaction for lactate.

The insoluble part of barium salts reduced Fehling's solution when warmed, although lacking Simon's reaction[11] for pyruric acid.

The ether extracts of undissolved part, acidified by sulphuric acid prior to the extraction, gave rhombohedric crystals of para–pyruric acid. After recystallisation from ether, this melted, begining at 70°C and ending at 85C; decomposed at 165°C, easily dissolved in water, alcohol as well as in ether but difficultly in benzol or ligroin. This acid reduced Fehling's solution when heated, although lacking Simon's reaction (l. c. nitroprussid Na in presence of alkali), converted into pyruvic acid when heated in alkaline medium, failed to form phenylhydrazone (Wolff. A. 305. 162).

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UEBER DIE QUANTITATIVE BESTIMMUNG DER PEPSINWIRKUNG

von

M. Matsuyama und H. Nakamura.

(Aus dem Laboratorium der Unionbrauerei bei Tokyo.)

(Eingegangen am Dez. 15, 1929.)

In vorliegender Arbeit ist das quantitative Verhältins der Pepsinverdauung behandelt und es sind darin neue Berechnungsformeln und Bezeichnungen für die Pepsinwirkung aufgestellt. Als pepsinpräparate sind zwei amerikanische Handels-Pepsine, die wir mit Pepsin A und B bezeichnen, und das Merk'sche Pepsin benutzt. Zunächst wurde die Eiweissverdauung durch Pepsin bei wechselnder Säurekonzentration und bei verschiedenen Temperaturen beobachtet, und zwar unter folgenden Versuchsbedingungen: 0.2 g. möglichst fein gepurvertes Eiereiweiss (Merk) ist mit Pepsin und 4 c.c. N.– Salzsäure in 40 c.c. Lösung bei 37° Cels. 1–3 Stunden digeriert. Die Säurekonzentration der Reaktionsmischung ist $P_H=1.75$. Die Menge der Salzsäure muss so gewählt werden, dass die Lösung die angegebene Säurekonzentration esgibt. Nach einer bestimmten Verdauungszeit wird die Lösung mit Natronlauge neutralisiert gegen Methyl-rot, man setzt 5 c.c. 1 Mol Kochsalzlösung hinzu, und das ganze wird in einem vorgekochten Wasserbad erhitzt. Die Lösung wird durch vorher abgewogenes Filtrierpapier filtriert und gewaschen, bis Cl frei ist, bei 105°C getrocknet, und dann wird die unverdaute koagulierbare Eiweissmenge bestimmt. Durch blinde Versuche mit Eiweiss ohne Pepsin und Pepsin ohne Substrat muss man die gewonnen verdaute Eiweissmenge korrigieren.

Eiweissverdauung mit wechselnder Pepsinmenge.

Das Ergebnis ist in folgender Tabelle zusammen gestellt:

Pepsin A

Tabelle I.

Pepsinmenge (g)	Verdaute Eiweissmenge (g) bei Verdauungszeit (Std.)		
	1	2	3
0.0001	0.0139	0.0197	0.0227
0.0002	0.0188	0.0272	0.0324
0.0004	0.0265	0.0395	0.0471
0.0006	0.0328	0.0453	0.0519
0.0008	0.0363	0.0503	——
0.0010	0.0409	0.0566	0.0657
0.0012	0.0447	0.0595	0.0684
0.0014	0.0493	0.0641	0.0732
0.0016	0.0515	0.0673	0.0752
0.0018	0.0536	0.0705	0.0779
0.0020	0.0560	0.0720	0.0812

Auf Grund der oben angegebenen Resultate wurden mehrere von anderen Autoren gegebene Reaktionskonstanten geprüft und gefunden, dass die Formel von Schütz und Huppert (Pflügers Arch. **80**, 470,1900) : $K=\frac{x}{\sqrt{t}}$, worin x=verdaute Eiweissmenge, t=Verdauungszeit ist, am besten mit uns übereinstimmt. Wir haben gefunden, dass sich die Verdauung auch mit ziemlicher Genauigkeit nach der Gleichung $K=\frac{x}{\sqrt[3]{t}}$ feststellen lässt. Folgende Tabelle zeigt die beiden K-Werte:

Fig. I.

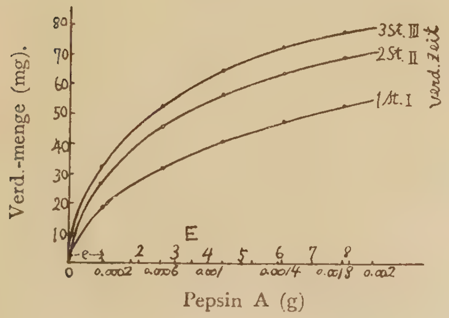


Tabelle II.

	$K=\frac{x}{\sqrt{t}}$			$K=\frac{x}{\sqrt[3]{t}}$			$K=\frac{x}{\sqrt[3]{t}}$
	bei Verd.-zeit (St.)			bei Verd.-zeit (St.)			Mittel.
Pepsinmenge (g)	1	2	3	1	2	3	2 u. 3
0.0001	0.0139	0.0139	0.0131	0.0139	0.0156	0.0157	0.0157
0.0002	0.0188	0.0192	0.0187	0.0188	0.0216	0.0224	0.0220
0.0004	0.0265	0.0279	0.0271	0.0265	0.0313	0.0326	0.0319

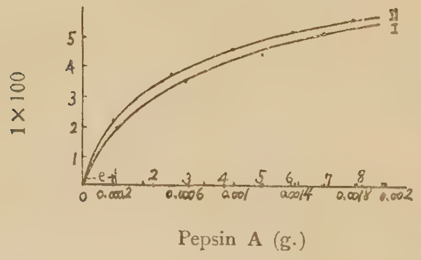
0.0006	0.0328	0.0320	0.0299	0.0328	0.0359	0.0359	0.0359
0.0008	0.0363	0.0355	0.0346	0.0363	0.0400	0.0416	0.0408
0.0010	0.0409	0.0400	0.0379	0.0409	0.0433	0.0455	0.0444
0.0012	0.0447	0.0420	0.0394	0.0447	0.0471	0.0474	0.0473
0.0014	0.0493	0.0453	0.0422	0.0493	0.0508	0.0508	0.0508
0.0016	0.0515	0.0476	0.0434	0.0515	0.0534	0.0521	0.0528
0.0018	0.0536	0.0498	0.0449	0.0536	0.0559	0.0540	0.0549
0.0020	0.0560	0.0509	0.0469	0.0560	0.0571	0.0563	0.0567

Aus vorstehender Tabelle ersieht man, dass die Formel $K=\frac{x}{\sqrt{t}}$ namentlich bei kleineren Fermentmengen besser angewandt wird, während bei grösseren Fermentmengen unsere Formel $K=\frac{x}{\sqrt[3]{t}}$ genauer zutrifft.

Die Differenz zwischen den $K=\frac{x}{\sqrt[3]{t}}$ Werten bei einstündiger und bei zweistündiger Verdauung ist verhältnissnässig gross, dagegen geben die Werte bei zwei-und dreistündiger Verdauungszeit die beste Konstante für den gesamten Bereich unserer Versuche. Wir haben danach zur Berechnung der Pepsinwirkung die Reaktionskonstante $K=\frac{x}{\sqrt[3]{t}}$ gebräucht.

Die K–Werte bei wechselnder Fermentmenge sind in Fig. II dargestellt. Der K–Wert bei einstündiger Verdauung ist gleich der Verdauungsmenge, welche man aus des Formel ersieht.

Fig. II.



In Fig. II zeigt Kurve I die K–Werte bei einstündiger Verdauung. Kurve II zeigt den Mittelwert von K bei zwei– und drei-stündiger Verdauung, der in Tabelle II in der achten Spalte angegeben ist.

In Fig. II ist der K–Wert zweckmässig verhundertfacht.

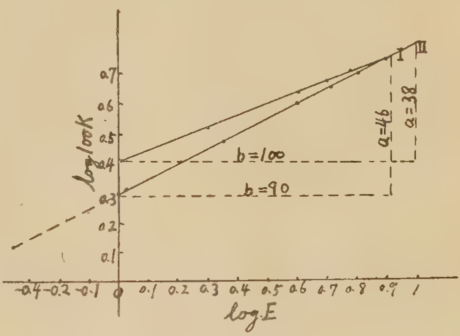
Zur Berechnung der Fermentwirkung haben wir als Pepsineinheit zweckmässigen Weise eine Enzymmengegewählt, für die sich unter den angegebenen Versuchsbedingungen bei einstündigen Verdauung eine Verdauungsmenge von 20 mg. ergibt.

In dem Koordinatsystem der K–Kurve wurde die Abszisse in gleiche Teile, deren Länge der Pepsineinheit gleich ist, geteilt,– in Fig II. ebenfalls angegeben.

Die Kurve stimmt mit der Formel 100 $K=AE^{n}$ überein, wobei K die Reaktionskonstante nach der Formel $K=\frac{x}{\sqrt[3]{t}}$, E die im Versuchspräparat enthaltene Pepsineinheit und A und n zwei Zahlenkonstanten darstellen.

Zur Prüfung der Formel und Berechnung der Konstanten logarythmiert man die Gleichung und erhält $\log 100K = \log A + n \log E$. Es ist dies die Gleichung einen Geraden, welche die $\log 100K$- Achse in der Höhe von $\log A$ schneidet und zur $\log E$- Achse in einem Neigungswinkel verläuft, dessen Tangent$=n$ ist. Man trägt also in ein Koordinatsystem als Abszisse die Werte von $\log E$, als Ordinaten die Werte von $\log 100K$ ein, wie dies in Fig. III geschehen ist : Trifft die Gleichung zu, so müssen die Punkte ungefahr auf einer Geraden liegen, wie Fig. III zeigt. Da, wie schon erwähnt, die K–Werte bei einstündiger Verdauung verhältnismässig stark abweichen von den K–Werten bei zwei-und dreistündiger Verdauung, haben wir die Gleichung für einstündige und für zwei-und dreistündige Verdauung getrennt aufgegeben. Log. A ist, wie erwähnt, gleich der Strecke, die die Gerade auf der log 100 K- Achse schneidet ; wie Fig. III zeigt, sind diese Werte 0.295 und 0.404, die zugehörenden Nummern also $A_1 = 1.9724$, $A_{2-3} = 2.5351$. Die Tangenten der Neigungswinkel ergeben sich als Quotienten der Strecke $a:b$, sie betragen $n_1 = \frac{46}{91} \doteqdot \frac{1}{2}$, $n_{2-3} = \frac{38}{100}$.

Fig. III.



Nun haben wir zwei Gleichungen, namentlich :

$$E_1 = \left(\frac{100\,K}{1.9724} \right)^2, \quad E_{2-3} = \left(\frac{100\,K}{2.5351} \right)^{\frac{100}{38}}.$$

E_1 bezeichnet die Einheit errechnet aus dem Ergebnis einstündiger, E_{2-3} die Einheit errechnet aus dem Ergebnis zwei-bis dreistündiger Verdauung. Man setzt die zwei Zahlenkonstanten in die oben angegebenen logarythmische Formel ein und berechnet damit E.

Nachstehende Tabelle zeigt E bei verschiedenen K-Werten beobachtet nach Fig. II und berechnet nach der Formel :

Tabelle III.

1–stdg. Verdauung.			2–3–stdg. Verdauung.		
$K \times 100$	E beobachtet	E berechnet	$K \times 100$	E beobachtet	E berechnet
1	0.4	0.26	2	0.7	0.53
2	1.05	1.02	3	1.57	1.56
3	2.2	2.29	4	3.18	3.32
4	4.0	4.08	4.4	4.09	4.22
4.5	5.2	5.16	4.8	5.22	5.36

5	6.5	6.38	5.1	6.18	6.28
5.5	8.1	7.72	5.3	6.96	6.97
			5.5	7.87	7.66

Giltig zwischen $100K=2$—5.2 $100K=2.9$—5.5

Nun bezeichnen wir das Mass der Pepsinwirkung ($P.f.$) derjenigen Anzahl von E, die in 1 g. Pepsin-Präparat enthalten ist, wie folgt : $P.f.=\frac{E}{\text{Präparat(g)}}$. Tabelle IV–VI gibt ein Beispiel, wie das Resultat der Pepsinwirkung nach unserer Fermel errechnet wird:

Tabelle IV : Pepsin A.

Pepsinmenge (g.) \ Verdauungsmenge (g.)	I 1 Std.	II 2 Std.	III 3 Std.
0.0002	0.0188	0.0272	0.0324
0.0004	0.0265	0.0395	0.0471
0.0006	0.0328	0.0453	0.0519
0.0008	0.0363	0.0503	0.0600
0.0010	0.0409	0.0566	0.0657
0.0012	0.0447	0.0595	0.0684
0.0014	0.0493	0.0641	0.0732
0.0016	0.0515	0.0673	0.0752
0.0018	0.0536	0.0705	0.0779
0.0020	0.0560	0.0720	0.0812

Pepsinmenge \ P. f. Verd. Zeit	I 1 Std.	II 2 Std.	III 3 Std.	IV
0.0002	4540	——	——	
0.0004	4500	4350	4625	I, II u. III Mittel.
0.0006	4600	4166	4166	
0.0008	4225	4150	4600	
0.0010	4290	4090	4660	
0.0012	4275	4258	4325	
0.0014	4457	4485	4450	
0.0016	4256	4444	4162	
0.0018	4105	4455	4066	
0.0020	——	4240	4085	
Mittel.	4360	4293	4348	4334
Die grössten Unstimmungkeiten gegen IV	6.1%	5.6%	6.7%	

Tabelle V : Pepsin B.

Peysinmenge (g.) \ Verdauungsmenge (g)	I 1 Std.	II 2 Std.	III 3 Std.
0.00008	0.0224	0.0330	0.0400
0.00016	0.0310	0.0445	0.0535
0.00024	0.0386	0.0532	0.0625
0.00032	0.0435	0.0595	0.0690
0.00040	0.0486	0.0650	0.0744

P. f. Verd. Zeit / Pepsinmenge (g.)	I	II	III	IV
	1 Std.	2 Std.	3 Std.	
0.00008	16333	——	——	
0.00016	15437	14937	16872	
0.00024	15958	15916	17000	
0.00032	15187	16062	16093	
0.00040	15175	16800	16200	
Mittle.	15618	15929	16541	15744
Die grössten Unstimmungkeiten gegen IV	5.3%	6.8%	6.5%	

Tabelle VI : Merksche Pepsin.

Verdanungsmenge (g.) / Pepsinmenge (g.)	I	II	III
	1 Std.	2 Std.	3 Std.
0.02	0.0261	0.0335	0.0403
0.04	0.0359	0.0505	0.0580
0.06	0.0433	0.0579	0.0674
0.08	——	0.0659	0.0753

P. f. Verd. Zeit / Pepsinmenge (g)	I	II	III	IV
	1 Std.	2 Std.	3 Std.	
0.02	87.5	——	——	
0.04	82.8	83.5	84.2	Mittel von I, II u. III
0.06	80.7	79.5	84.0	
0.08	——	84.5	83.4	
Mittel	83.6	82.3	83.4	83.1
Die grössten Unstimmungkeiten gegen IV	5.3%	4.3%	1.3%	

Zusammenstellung.

1) Das quantitative Verhältnis der Eiweissverdauung durch Pepsin wurde untersucht, und es wurden neue Berechnungsformeln und Bezeichnungen aufgestellt.

2) Versuchsbedingungen sind folgende :

0.2 g. gepurvertes Eiweiss, 4 c.c. N. HCl Lösung, Pepsinlösung und Wasser, Gesamtvolumen$=$40 c.c. ($P_H=1.75$).

3) Die Formel $P.\ f.=\frac{E}{\text{Präparat (g)}}$ gibt die Pepsinwirkung an.

E wird nach folgenden Formel berechnet :

bei 1 stündigen Verdauung : $E_1=\left(\frac{100\ K}{1.9724}\right)^2$

bei 2-3-stündigen Verdauung : $E_{2-3}=\left(\frac{100\ K}{2.5351}\right)^{\frac{100}{38}}$

wobei $K=\frac{x}{\sqrt[3]{t}}$, $x=$verdaute Eiweissmenge (g.), $t=$Verdauungszeite (Std.) ist.

Bei einstündiger Verdauung ist K gleich der verdauten Eiweissmenge.

4) Bei unseren Versuchen mit dreierlei Pepsin-Präparaten ergaben sich—bei 49 Bestimmungen—die grössten Unstimmungkeiten gegen die Mittelwert 6.8%.

ON THE CHANGE OF SULPHUR LINKAGE IN HORDEIN DENATURED BY ETHYL ALCOHOL.

By

Dr. E. Takahashi and K. Shirahama.

(Received Feb. 20th., 1929).

The solution of hordein in 94 per cent ethyl alcohol was heated on the steam bath of 75°C and the hordein was fractionally separated at the intervals of 8, 20 and 32 hours, the coagulum part by filtration and the emulsoid part by precipitation with NaCl.

These products obtained from above treatments, were analised of total sulphur (by Denis-Benedict's method) and cystine sulphur (by Okuda's method).

Analytical date were as follows :–

Sample	Cystine S.	Total S.	Ratio Cys. S : Tot. S
Original hordein	0.139%	0.628%	22 : 100
8. C.	0.340	0.597	57 : 100
8. E.	0.226	0.609	37 : 100
20. C.	0.435	0.621	68 : 100
20. E.	0.319	0.675	47 : 100
32. C.	0.663	0.771	86 : 100

C. ……Coagulum part. E. ……Emulsoid part.

From above results, it is recognised that the sulphur linkage in hordein is changed and –S–S– linkage may appear on the proceeding of denaturation by ethyl alcohol.

UEBER DIE WACHSARTIGE SUBSTANZ DES KOKON-SEIDENFADENS.

von

OKU MASAMI.

(*Aus dem chemischen Laboratorium, Gunze Gesellschaft zur Herstellung von Rohseiden, Ayabe, Kyōto-hû, Japan.*)

(Eingegangen am Feb. 21., 1929.)

Die wachsartige Substanz von Kokonseidenfäden, deren eingehende Untersuchungen bis heute noch nicht gemacht worden sind, hat nicht nur eine physiologische Bedeutung für die Seidenwürmer, sondern übt auch einen praktischen Einfluss auf Rohseiden-industrien aus. Nach-folgende Untersuchungen beschäftigen sich hauptsächlich mit den chemischen Beschaffenheiten dieses Kokonwachses.

Kurze Zusammenfassung von Versuchsresultaten.

Kokonschichten von Bombyx mori wurden mit Aether extrahiert und eine rohe wachsartige Substanz erhalten. Die chemischen Eigenschaften derselben konnten wie unten ausgeführt zusammengefasst werden.

1.) Das Rohwachs.

Es ist ziemlich orange gefärbt wegen der Beimengung von Kokonpigment. Die getrocknete Substanz ist leicht purverisierbar. Sie bestand aus ca. 80% in siedenden Alkohol löslichen Teilen und 20% in Alkohol unlöslichen Teilen. Es schmilzt bei 65° (unkorr.). Die Elementaranalyse zeigte, C% 77.9, H% 13.2, O% 8.9.

Die qualitative Versuchen zeigten keinen Glyzerin und Stickstoff.

2.) Das reine Wachs.

Das Rohwachs wurde durch einmaliges Umkristallisieren aus siedendem Alkohol gereinigt und frei von Pigment gemacht. Alle unten erwähnten Versuche wurden mit diesen reinen Wachsen ausgestellt. Es bestand aus 63.14% unverseifbarer Substanz und 36.86% von Fettsäuren. Es zeigte,

Schmelzpunkt 68°∼69°(unkorr.), Säurezahl 13.42, Verseifungszahl 110.77, Iodzahl 4.49, Elementarzusammensetzung C% 79.4, H% 13.9, O% 6.7.

3.) Die unverseifbaren Substanzen.

Reines Wachs bestand aus 81.92 Teilen höheren Alkohols und 18.08 Teilen Kohlenwasserstoffen. Kein Vorhandensein von Sterine wurde bestätigt, durch gelegentliche Farbenreaktionen von Sterine. Es schmilzt bei 84° (unkorr.). Die Elementaranalyse ergab, C% 81.6, H% 14.6, O% 3.8. Beim Erwärmen mit Essigsäureanhydrid ergibt sich zum grössten Teile eine Lösung und scheidet sich beim Erkalten : aber eingeringer Teil löst sich nicht und scheidet als ölige Tropfen aus siedendem Essigsäureanhydrid aus. Erstere Eigenschaften zeigten das Vorhandensein von höheren Alkoholen und die letztere das von Kohlenwasserstoffen.

4.) Die Alkohole des unverseifbaren Bestandteils.

Die Wachsalkohole und Kohlenwasserstoffe wurden voneinander durch Kochen mit Essigsäureanhydrid getrennt. Die acetilierten Alkohole zeigten,

Schmelzpunkt 67° (unkorr.), Verseifungszahl 127.7, Elementarzusammensetzung C% 79.35, H% 13.25.

Vgl.	C%	H%	F.P.	Verseif.-zahl
Cerylacetat	79.16	13.30	65°	132.3
Myricylacetat	79.92	13.40	70°	116.7

Auf diesen Daten, kann die Beimengung von Cerylalkohol und Myricylalkohol in diesen Wachsalkohol vermutet werden.

5.) Kohlenwasserstoff des unverseifbaren Bestandteils.

Die Kohlenwasserstoffe wurden gereinigt zuerst durch mehrfaches Kochen mit dem Gemische von gleichen Volumen von Amylalkohol und rauchenden Salzsäure. Es ist unlöslich in siedendem Essigsäureanhydrid und Alkohol, löslich in warmen Aether, Benzol und Chloroform. Es schmilzt bei 68° (unkorr.). Die Elementarzusammensetzung ist, C% 84.11, H% 14.84.

Vgl.	C%	H%	F.P.
Hentriakontan $C_{31}H_{64}$	85.22	14.78	68°

Es addiert sich nicht Brom. Wir vermuteten deswegen, dass der Wachskoholenwasserstoff hauptsächlich aus Hentriakontan bestand.

6.) Die Fettsäuren vom Kokonwachs.

a) Die Menge der gesamten Fettsäuren kann aus der Differenz von reinem Wachs und dessen unverseifbaren Bestandteil erhalten werden : es betrug also 36.86% des Wachses. Wir berechneten aus der Verseifungszahl und den gesamten Fettsäuren,

Neutralisationszahl 300.52, Molekulargewicht 186.71.

b) Die Hehnerschen Zahlen betrugen 13.40%. Die in Wasser unlöslichen Fettsäuren zeigten folgende Eigenschaften:

Schmelzpunkt 85° (unkorr.), Neutralisationszahl 126.74, Molekulargewicht 442.72, Elmentarzusammensetzung C% 79.96, H% 14.04.

Vgl.	C%	H%	F. P.	Mol.-gew.
Melissinsäure	79.56	13 37	88.5°	452.48

Deshalb kann das Vorhandensein von Melissinsäure vermutet werden, jedoch muss die Anwesenheit von populären Fettsäuren z. B. Palmitin- und

Stearinsäure als zweifelhaft angesehen werden.

c) Die in Wasser löslichen Fettsäuren betrugen 63.65% der gesamten Fettsäuren. Die saure Lösung, welche von in Wasser unlöslichen Fettsäuren getrennt wurde, hat einen stark riechenden, unangenehmen Geruch und bewies deutlich das Vorhandensein von niedrigen Fettsäuren.

Wir berechneten, Neutralisationszahl 399.76, Molekulargewicht 140.36.

Aus den vorhergehenden Tatsachen fanden wir, dass diese Kokonwachse viel Ähnlichkeit mit den Bienen-wachsen haben.

ON THE SIMPLIFIED, SATURATED CALOMEL ELECTRODE. REPORT II.

By

ARAO ITANO, PH. D.

(Received Feb. 2nd., 1929).

This paper deals with a simplified, portable form of saturated calomel electrode which is arranged in an ordinary vessel and has a certain advantages over the Ostwald vessel, and serves the purpose satisfactorily.

The mixture of mercury, calomel and saturated potassium chloride is placed in an ordinary pyrex glass cup in which a platinum electrode and one end of an agar bridge are placed properly through a rubber stopper, and the other end of agar bridge is immersed in the saturated KCl solution by which the chain is formed.

The calomel electrode thus prepared gives very good results in comparison with those obtained using the standard N/10 KCl calomel electrode. Some of the results are shown in Table I.

Tabele I.

pH determined by the simplified and standard Calomel Electrode.

Experimentor.	Temp. °C	pH obtained*	
		Simplified sat. electrode.	Standard eelctrode.
I.	14.	7.31	7.24
A.	〃	7.29	7.25
M.	〃	7.31	7.28

* Phosphate buffer solution was used.

The results indicate that they are close enough for ordinary experimental purpose.

In this arrangement, the calomel electrode is well protected from contamination by the testing solution and the deposition of potassium chloride crystals which is formed ordinarily can be avoided. Consequently it can be used very efficiently and economically for a long time.

ÜBER EIN STÄRKEVERFLÜSSIGEN DES ENZYM IN HEFEAUTOLYSATEN.

von

S. NISHIMURA.

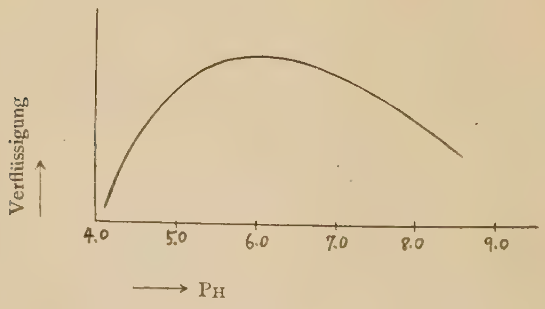
(Eingegangen am Feb. 22., 1929)

50 g. gewaschene und abgesaugte Bierhefe wurden unter Zusatz von 5 ccm. Toluol plasmolysiert und unter häufigem Umrühren einige Stunden bei Zimmertemperatur (ca. 25°C) autolysiert. Nachdem die Hefe dickflüssig geworden war, wurden 200 ccm. Wasser zugesetzt und unter häufigem Umschütteln wurde noch 24 Stunden extrahiert und je 5 ccm. des Filtrates zu den einzelnen Versuchen verwendet.

Die Einwirkung der Hefelösung wurde immer in 1%igen verkleisterten Stärkelösung bei 20–21°C vorgenommen und es wurde gefunden, dass die Hefelösung ein stärkeverflüssigendes Enzym enthält und die Stärke bei PH=6.2 in ca. 7 Stunden verflussigt.

Das Enzym hat ein Optimum der PH bei 6.0–6.2, die optimale Temperatur liegt bei 30–35°C und es wird durch 10 Minuten langes Erhitzen auf 50°C fast ganz zerstört.

Folgende Tabelle zeigt die Stärkeverflüssigung als Funktion der PH:



Das Enzym greift das Amylopektin langsam an und löst es auf, d.h. verflüssigt die Stärke.

Ich schlage die Bezeichnung Amylopektinase dafür vor.

Die Eigenschaften des Enzyms sind ähnlich denen der Maltase. Die Amylopektinase ist jedoch erst bei PH=4.0 unwirksam im Gegensatz zu der Unwirksamkeit der Maltase bei PH=4.5, die erstere ist also etwas säurebeständiger. Man kann in der Hefelösung die Amylopektinase von der Maltase dadurch trennen, dass die Maltase bei 25°C nach etwa einer Woche, bei 20°C nach etwa zwei Wochen zerstört wird, dagegen die Amylopektinase unter diesen Umständen noch sehr wirksam bleibt.

Die Unvollkommenheit des Stärkeabbaues durch die Amylasen besonders durch die Gersteamylase beruht auf der Schwierigkeit der Stärkeverflüssigung. Es ist deshalb anzunehmen, dass die Verzuckerung der Stärke durch die Hefelösung beschleunigt würde. Die Amylaselösung wurde unter Zusatz von Hefelösung der Stärke zugesetzt. Die Maltasewirkung der maltasefrei gewordenen Hefelösung wurde sorgfältig in einem Versuchsansatz mit Maltoselösung geprüft. Folgende Tabelle zeigt einige Beispiele:

	Verflüssigung	Spaltung in Tagen			
		1	3	6	12
Gersteamylase	ca. 2 Tage	Blauviolett	Violett	Violettrot	Rot
G.A.+Hefelösung	ca. 7 Stunden	Violett	Rot	Rotbraun	Gelb

	Verzuckerung in Tagen				% Maltose
	1	3	6	12	
Gersteamylase	0.603 g.	0.644 g.	0.677 g.	0.718 g.	68.0
G.A.+Hefelösung	0.782	0.878	0.931	0.975	88.0 (als Glukose)
G.A.+H.L. (Maltase frei)	0.693	——	0.852	0.945	90.0
Malzamylase	0.774	——	0.824	0.857	81.0
M.A.+H.L. (Maltase frei)	0.866	——	0.933	0.984	93.2

Durch diese ist bewiesen worden, dass das stärkeverflussigende Enzym der Hefe die Verzuckerung der Stärke durch die Amylasen besonders durch die Gersteamylase bedeutend beschleunigen kann.

昭和三年十二月七日印刷
昭和三年十二月十日發行

東京帝國大學農學部內
發行兼編輯者　松山芳彥

東京帝國大學農學部內、日本農藝化學會
印刷者　河村秀兼

東京帝國大學農學部內
印刷所　農藝化學敎室印刷所

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PLATE I